N° 32. **P. S. Chen** and **F. Baltzer,** [1], Bern. — Investigations on the determination of the adhesive glands in *Bombinator pachypus* (xenoplastic transplantations). (With 9 figures in text.)

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The adhesive glands in *Bombinator* larvae begin to differentiate in the neural plate stage. They consist of a pair of flat ovoid-shaped discs situated on the ventral side of the head region posterior to the mouth (Fig. 1). Histologically each disc contains an outer layer of cylindrical secretion cells and an inner thin layer of ordinary epidermal cells (Fig. 2).

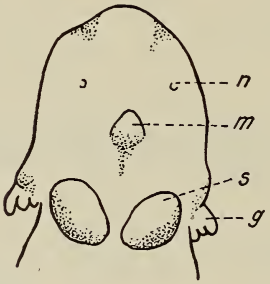

FIG. 1.

The normal location of suckers in a young *Bombinator* larva (approx. POLLISTER and MOORE stage 20), g, gill; m, mouth; n. nostril; s. sucker. × 12.

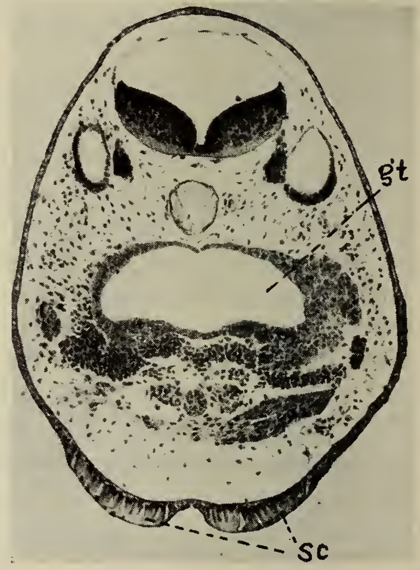

FIG. 2.

Cross section through the sucker region of a *Bombinator* larva (POLLISTER and MOORE stage 21), showing the differentiation of secretion cells (sc.), gt, gut. × 50.

YAMADA (1933) studied the determination of these glands during gastrulation in *Rana nigromaculata* by methods of isolation (interplantation of gastrula ectoderm into the larval body-cavity of the same species) and homoplastic heterotopic transplantation of the presumptive sucker area into the trunk. According to him the determination of the sucker began during gastrulation and became settled in the young neurula. If the material was taken from old gastrula, the implants in the trunk region produced suckers. However if he used young gastrula ectoderm for transplantation, the formation of sucker was not observed. Similar conclusions were reached by him in a later experiment of explantation (YAMADA 1938).

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A more definite localization of the sucker area during gastrulation and neurulation was not considered closely by him. The following experiments are dealing with this point which concerns at the same time the problem of homology between the sucker of Anura and the balancer of Urodele larvae (F. BALTZER and P. S. CHEN 1951). Bombinator material was transplanted into Triton hosts. In all experiments the observations on the living material were completed by the examination of sectioned embryos.

I. TRANSPLANTATION IN NEURULA STAGE.

A. *Head epidermis from Bombinator neurula into Triton neurula.*

A piece of epidermis from the rostro-lateral head region of a *Bombinator* neurula was transplanted orthotopically into the corresponding area of a *Triton* neurula. The operation sketch is given in Fig. 3. The larvae were cultured for 2-4 days.

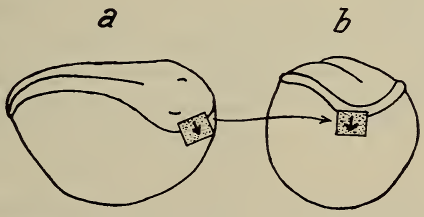

FIG. 3.

Orthotopic transplantation of head epidermis from a young *Bombinator* neurula (*a*) to a *Triton* neurula (*b*). In the neural fold area the transplant involved only the covering epidermis.

In 22 out of 28 cases suckers were formed, all of which were however smaller than normal ones. In the majority of cases they were located at the posterior-ventral edge of the implant (Fig. 4).

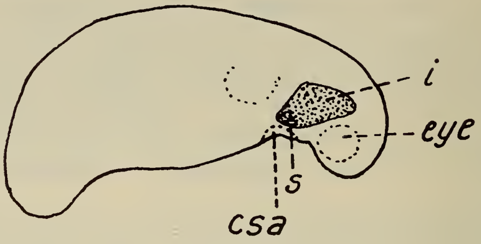

FIG. 4.

The development of sucker at the posterior-ventral edge of orthotopically transplanted head epidermis, 2 days after operation stage. The area indicates that the orientation of the transplant has been maintained. (Protocol XIV, 6). csa, corresponding sucker area of a *Bombinator* larva; i, implant; s, sucker.

In sections the suckers showed normal differentiation of secretion cells. In Fig. 5 the locations of 18 suckers in the *Triton* hosts are summarized. Each point represents the sucker in one experiment. The majority of them were situated lateral to and outside of the area (csa) where the sucker in *Bombinator* normally appears.

In 12 donors the development of the sucker on the operation side was followed. It was found that the suckers were abnormally small, and that always the lateral part of this organ was lacking. Thus we have to conclude: Firstly, these sucker was completely determined in the young neurula stage at the time of operation. But the transplant did contain only a part of the anlage, and the remainder was left in the donor. Secondly, the presumptive sucker area covers the lateral field of the

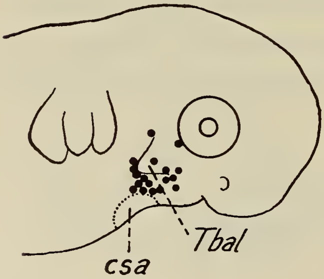

FIG. 5.

A topographical summary of the location of suckers developed in *Triton* hosts (orthotopic transplantations) All points are smaller than the actual sucker size. The dotted line indicates the corresponding sucker area of a *Bombinator* larva (csa). Tbal, *Triton* balancer.

head and extends more dorsally than the sucker formed later. Thirdly, the partial glands, as reported by YAMADA, were unable to regulate the missing part.

In the 6 negative cases the transplants apparently did not include the presumptive sucker area.

B. *Head epidermis from* Bombinator *neurula into* Triton *neurula, with the implant turned 180°.*

In 7 out of 9 cases a sucker was formed at the dorsal edge of the implant. Two cases remained doubtful. In all cases the size of the sucker was again abnormally small. The results of experiments A and B are as follows: They confirm, in agreement with YAMADA, that the sucker is definitely determined and localized in the young neurula; and further, that its field in this stage extends in a latero-dorsal direction.

C. *Head epidermis from* Bombinator *neurula into the trunk region of* Triton *neurula.*

The rostro-lateral head epidermis from a *Bombinator* neurula was transplanted heterotopically into the trunk region of a *Triton* neurula. In 8 cases distinct suckers developed. An analysis of the development of suckers in donors showed that in all these 8 cases the *Bombinator* larvae had abnormally small suckers on the operation side.

D. *Trunk epidermis from* Bombinator *neurula into the head region of* Triton *neurula.*

In these experiments it was an expected result that the sucker did not appear. The results of C and D correspond to similar heterotopic but homoplastic experiments of YAMADA.

II. TRANSPLANTATION FROM BOMBINATOR GASTRULA INTO TRITON NEURULA.

A. *Gastrula ectoderm from* Bombinator *into the head region of Triton neurula.*

The operation sketch is shown in Figs. 6 *a* and *b*. The transplant consisted of only the outer layers of the gastrula ectoderm. It was taken from the presumptive anterior belly area. The *Triton* larvae were cultured for a period of 5-7 days. In the majority of cases a large part of the transplant differentiated into epidermis, and a smaller part of it migrated into the interior of the host as visceral mesectoderm material (compare HOLTFRETER 1933). Among the 20 experiments distinct suckers were formed in 12 cases, all of which had a location again at the ventral edge of the

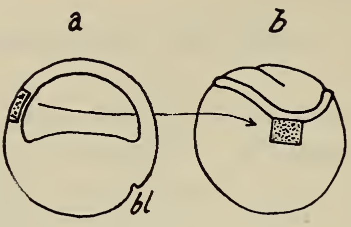

FIG. 6.

A piece of *Bombinator* ventral gastrula-ectoderm *(a)* was transplanted into the rostrolateral head region of a *Triton* neurula *(b)*. bl, blastopore.

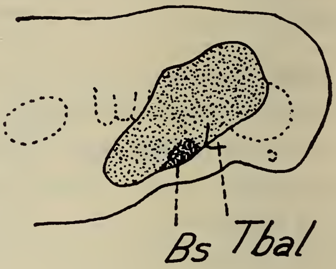

FIG. 7.

A case 5 days after operation, sketch from living larva. Bs, *Bombinator* sucker; Tbal, chimeric *Triton* balancer. (Prot. LXX, 1.)

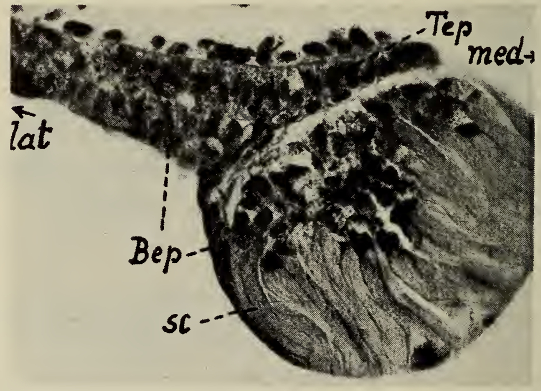

FIG. 8.

Bombinator epidermis with sucker at its ventral edge (Protocol XVIII 1 T). Bep, epidermal layer from *Bombinator*; lat, lateral; med, medial; sc, secretion cells; Tep, epidermal layer from *Triton*. × 160.

implant area as in groupe I A. In the remaining 8 cases the formation of this organ was either doubtful or not observed.

Figs. 7 represents an outside view of a living larva 5 days after operation. Fig. 8 shows the histological differentiation of the sucker which is normal. A topographical summary similar to that given in Fig. 5 is presented in Fig. 9 for group II A. All suckers were located at the lateral side in the head region of the *Triton* host, and again outside of the area where they in *Bombinator* normally appear. Two similar cases with lateral but more posterior locations have been observed by HOLTFRETER (1935, p. 475).

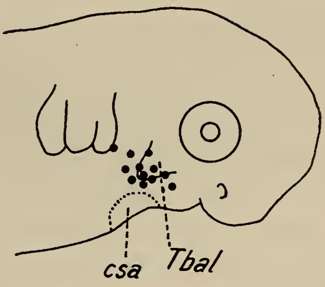

FIG. 9.

A topographical summary of sucker locations in *Triton* hosts (*Bombinator* gastrula ectoderm into *Triton* neurula). Indications same as in Fig. 5.

B. *Gastrula ectoderm from* Bombinator *into trunk region of* Triton *neurula.*

The transplant was taken from the gastrula in the same way as in section II A, except in the present case it was grafted into the trunk region. About 4 days later in most cases the transplant was found to be located in a region posterior-ventral to the anterior limb bud. In all 14 experiments no sucker formation was observed. The negative results have been confirmed by the study of sectioned embryos.

The results of experiments II A and B are as follows: They extend the famous findings of SPEMANN and SCHOTTÉ (1932). These authors transplanted ectoderm of anuran gastrula into *Triton* gastrula, and sucker developed in the transplant. The present study showed that the same happened when *Bombinator* gastrula ectoderm was transplanted into *Triton* neurula. Thus the determination, which in *Bombinator* is provided at the gastrulation period, can be furnished in the xenoplastic experiment during the neurulation of the *Triton* host as well. Furthermore, section II B confirm the findings of YAMADA, according to whom the sucker determination is still absent in the ectoderm of the young gastrula.

III. EXPERIMENTS ON EXOGASTRULATION.

SPEMANN and SCHOTTÉ could not give any indication about the inductor itself. YAMADA (1938) concluded from his own experiments that the anterior end of the neural plate and the head gut were the two main inducing factors. HOLTFRETER (1935) assumed the presence of an induction center posterior to the mouth and the extension of the induction process in the form of a gradient from this center to the dorsal side. WAGNER (1949) was in favour of at least a partial determination by the mesectoderm of the mandibular arch. The following experiments on exogastrulae deal with this question.

Bombinator embryos in middle or late blastula stage were carefully removed from the egg membrane, and cultured further in either HOLTFRETER-or 0,35% NaCl solution. In total 23 blastulae were treated and kept in the above mentioned solutions for 2-7 days before they were fixed. Embryos of the same age but retaining the egg membranes served as controls.

The development of suckers in the treated larvae varied extremely, ranging from cases with almost normal suckers to those in which the formation of sucker was entirely absent. No correlation can be seen between the degree of exogastrulation and sucker differentiation. Thus our material so far shows only one fact, i. e. that exogastrulation is in many cases correlated to abnormal sucker formation. Meanwhile the development of gut and brain in these larvae is usually abnormal. But whether gut or brain abnormality is more involved with sucker formation till now cannot be made out.

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Nº 33. **Robert Schenk**, Zürich. — Ueber Defektoperationen an der dorsalen Urmundlippe junger Gastrulae von *Triton alpestris.* Mit 5 Abbildungen im Text.

(Anatomisches Institut der Universität, Zürich).

Defektoperationen an der dorsalen Urmundlippe von Amphibiengastrulae wurden schon von verschiedenen Autoren ausgeführt. Diese Region der Gastrula, die sich vom Urmundgrübchen fächerartig in der Richtung gegen den animalen Pol ausdehnt, nimmt bei der Ausbildung der embryonalen Grundgestalt eine Sonderstellung ein. Aus ihr entsteht während der Gastrulation das Urdarmdach, aus welchem sich die Achsenorgane des Embryo herleiten. Gleichzeitig induziert dieses Material in dem darüberliegenden Ectoderm die Medullarplatte. Werden während der Gastrulation Teile der dorsalen Urmundlippe entfernt, dann treten im Urdarmdach Ausfälle auf, die meistens von entsprechenden Defekten in der Medullarplatte begleitet sind (Lehmann 1926, 1928, 1938, Töndury 1937, Shen 1938). Wird diese Operation zu Beginn der Gastrulation ausgeführt, dann werden hauptsächlich die Kopforgane betroffen, und es kommt zur Entwicklung von Keimen, die eine mehr oder weniger ausgeprägte Mikrocephalie erkennen lassen. Daneben zeigen aber gerade die vorderen Ab-